

**AMENDMENT TO THE SPECIFICATION:**

Please replace the paragraph at page 35, line 1 through line 11 with the following amended text:

**Table 1:** Primers used for the PCR amplification of fragments of AC005038.

Primer name	Primer sequence	SEQ ID NO:
PNHsp1	5' - CGGTGCACTCAGGTGATTCCTCC - 3'	SEQ ID NO: 31
PNHsp2	5' - GGCAGCAAACCCATTATACTGGAACC - 3'	SEQ ID NO: 32
PNHsp3	5' - CGCTGGTGCAGTGAAGAGCC - 3'	SEQ ID NO: 33
PNHsp4	5' - CTGCACCCCCATCTGCTCTTCC - 3'	SEQ ID NO: 34
PNHap1	5' - GCAGGAAGAGCCACCGTAAGG - 3'	SEQ ID NO: 35
PNHap2	5' - CCAGTCTGCAAAGCCCTGGAGC - 3'	SEQ ID NO: 36

Please replace the paragraph beginning at page 41, line 26 and ending at page 43, line 16 with the following amended text:

To evaluate the existence of different mRNA transcripts for Enovin, RT-PCR experiments were performed using primers situated at the 5' end of the Enovin coding sequence just 5' of a possible upstream ATG start codon (primer PNHsp5 [5'-GCA AGC TGC CTC AAC AGG AGG G-3' (SEQ ID NO: 37)] and nested primer PNHsp6 [5' -GGT GGG GGA ACA GCT CAA CAA TGG-3' (SEQ ID NO: 38)] at the 3' end (primer PNHap1 and nested primer PNHap2 [see Table 1]. Experiments were performed on human multiple tissue cDNA panels (Clontech MTC panels I and II), on a fetal heart cDNA library (Clontech) and on cDNA derived from human cerebellum, hippocampus or frontal cortex (Masure et al., 1998). Primary PCR reactions were performed with primers PNHsp5 and PNHap1 under GC-rich conditions (Advantage GC-PCR kit, Clontech) for 30 cycles 95°C - 30s, 60°C - 30s, 72°C - 1 min) as described. Nested PCR reactions were performed on 1

µl of the primary PCR product using primers PNHsp6 and PNHap2 under the same conditions of 30 cycles. Resulting PCR products were analysed on a 1.5% agarose gel and ranged in size from ± 350 bp to ± 800 bp. Several bands were purified from the gel and the PCR fragments sequenced directly. Some purified PCR products were also cloned in vector pCR2.1-TOPO (TOPO-TA cloning kit, Invitrogen) and then sequenced. Sequence analysis confirmed the existence of different mRNA molecules containing Enovin sequence. The obtained fragment sequences were compared with the genomic Enovin sequence. This allowed us to identify several possible 5' and 3' splice sites in the genomic sequence (Figure 21) (SEQ ID NOS: 11 through 15). All these splice sites corresponded to the consensus sequences for donor and acceptor sites (Senapathy, P. Shapiro, M.B. & Harris, N.L. (1990)) splice junctions, branch point sites, and exons: sequence statistics, identification, and applications to genome project. Methods Enzymol. 183, 252-278). The different Enovin splice variants identified and their presence in different human tissues are summarized in Figure 22. Only two of the 5 sequenced transcripts yield functional Enovin protein upon translation from the ATG start codon. These two transcripts code for proteins of 228 or 220 amino acids, respectively with predicted signal peptides of 47 and 39 amino acid residues. The predicted protein sequences of these two variants are shown in Figure 23 (long variant) (SEQ ID NO.: 9) and Figure 24 (short variant) (SEQ ID NO.: 10). The long variant can be deduced from the DNA sequence of Figure 21 (SEQ ID NO.: 8) by splicing out the first intron at locations 5'-1 and 3'-1 and the second intron at 5'-2 and 3'-3 (SEQ ID NO.: 12). Upon translation of the open reading frame, the predicted protein sequence of Figure 23 is obtained. The shorter variant can be deduced from the DNA sequence of Figure 21 by splicing out the first intron at locations 5'-1 and 3'-2 and the second intron

at 5'-2 and 3'-3. Upon translation of the open reading frame, the predicted protein sequence of Figure 24 is obtained.

Please replace the paragraph beginning at page 44, line 14 and ending on page 45, line 26 with the following amended text:

A 414 bp PCR fragment was amplified from human genomic DNA with primers PHNsp4 and PHNap2 (Table 1) and cloned in vector pCR2.1-TOPO using TA-cloning (Invitrogen). The sequence of the insert was confirmed by sequence analysis. One clone containing an insert with Enovin consensus sequence (clone 36) was used for subsequent construction of an expression plasmid. Two primers were designed containing appropriate restriction sites at their 5' ends. Forward primer PNHexp-spl (5'- GCG **GAT CCG** GCT GGG GGC CCG GGC A -3' (SEQ ID NO: 39)) contained a BamHI restriction site (underlined in bold, italics) and reverse primer PNHexp-apl (5'- GCC **TCG AGT** CAG CCC AGG CAG CCG CAG G -3' (SEQ ID NO: 40)) contained a XhoI restriction site (also underlined in bold, italics). Using these primers, the 343 bp fragment coding for mature Enovin (position 81 to 422 in Figure 1) was amplified from clone 36. The PCR reaction was performed in a total volume of 50 µl, containing 1x GC cDNA PCR reaction buffer, 0.2 mM dNTP, 1 M GC-MELT™ (, 200 nM of primers PNHexp-spl and PNHap1, 1 µl of Advantage KlenTaq polymerase mix and 10 ng of plasmid DNA from clone 36. Samples were heated to 94°C for 5 min and cycling was done for 45 s at 94°C, 1 min at 58°C and 30 s at 72°C for 25 cycles, with a final step of 7 min at 72°C. The resulting 50 µl product was purified using Qiaquick PCR purification kit (Qiagen) and the DNA eluted in 30 µl. 25 µl of this purified product was then digested in a 30 µl reaction with 10 U of BamHI and 10 U of XhoI in 1x buffer B (Boehringer Mannheim) for 1 h at 37°C. After electrophoresis in a 1% (w/v) agarose gel in 1x TEA buffer (40 mM Tris-acetate, 1 mM EDTA (sodium salt), pH 8.3), the expected 353 bp band was excised from the gel and purified

using the Qiaquick gel extraction kit. The resulting fragment was ligated in the vector pRSET B (Invitrogen) linearised with BamHI and XhoI. The insert of the resulting plasmid construct (hEVNmat/pRSETB) was confirmed by complete sequence analysis. The resulting construct codes for a 146 amino acid protein with a predicted molecular mass of 15704 Da including an NH<sub>2</sub>-terminal 6x His-tag fused in frame to the mature Enovin coding sequence. The NH<sub>2</sub>-terminal amino acid sequence of the resulting protein is thus

MRGS**HHHHHH**GMASMTGGQMQMRDLYDDDDKDP**AGGPGS** (SEQ ID NO: 41)  
(mature Enovin sequence in bold, 6x His tag underlined in bold, italics).

Please replace the paragraph beginning at page 47, line 10 and ending on page 48, line 1 with the following amended text:

A 3.3 kb fragment of the Enovin gene was amplified from cerebellum cDNA using primers EVN(7)-sp1 (5'- TTC GCG TGT CTA CAA ACT CAA CTC CC -3' (SEQ ID NO: 42)) and PNHap1 (5'- GCA GGA AGA GCC ACC GGT AAG G -3' (SEQ ID NO: 43)) designed on the sequence of EMBL accession number AC005038. The PCR reaction was performed in a total volume of 50 µl, containing 1x Expand Long Template PCR reaction buffer (Boehringer Mannheim), 0.5 mM dNTP, 1 M GC-MELT+ (Clontech Laboratories), 400 nM of primers EVN(7)-sp1 and PNHap1 and 1 µl of cerebellum DNA. After an initial 2 min at 94°C, 0.75 µl of Expand Long Template polymerase (Boehringer Mannheim) was added and cycling was done for 10 s at 94°C, 30 s at 58°C and 3 min at 68°C for 10 cycles. Then, 20 additional cycles were performed increasing the extension time at 68°C with 20 s every cycle. A final 7 min at 68°C were also included. The resulting 3.3 kb fragment was purified after electrophoresis in a 0.8% agarose/TAE gel and cloned in vector pCR2.1-TOPT using TA-cloning (Invitrogen). Complete sequence analysis of the 3.3 kb insert of one clone confirmed that the obtained cDNA sequence corresponded to the genomic sequence in the EMBL

database (accession number AC005038). No introns were spliced  
our in the cDNA obtained from cerebellum cDNA.

Please replace the paragraph beginning at page 66, line 12 through line 18 with the following  
amended text:

**Primer/probe design**

A pair of primers and a TaqMan probe were designed to  
amplify a specific sequence from Enovin

Primer 1: 5' ACGGTTCTCCAGGTGCTGT 3' (SEQ ID NO: 44)

Primer 3: 5' TGCTGCCGACCCACG 3' (SEQ ID NO: 45)

Probe 5: 5' CTACGAAGCGGTCTCCTTCATGGACG 3' (SEQ ID NO: 46)

Please replace the paragraph beginning at page 66, line 20 through line 29 with the following  
amended text:

In addition a pair of primers and a TaqMan probe were  
designed which span an intron and amplify a portion of the  
human GAPDH gene

**Primer 2:**

5' CAGAGTTAAAGCAGCCCTGGT 3' (SEQ ID NO: 47)

**Primer 4:**

5' GAAGGTGAAGGTCGGAGTCAAC 3' (SEQ ID NO: 48)

**Probe 6:**

5' TTTGGTCCGTATTGGGCGCCT 3' (SEQ ID NO: 49)

Appl. No.: 09/357,349  
Response dated February 23, 2004  
Response to Notice to Comply date January 21, 2004

Express Mail Label No.: EK897085215US  
Date of Deposit: February 23, 2004

**AMENDMENTS TO THE DRAWINGS:**

The attached sheets of drawings (see Appendix) include changes to Figure 1, Figure 2, Figure 3, Figure 21, Figure 23, and Figure 24. Where appropriate, sequence identifiers have been added to each of the above-referenced figures in compliance with the requirements of sequence rules 37 CFR § 1.821 through 1.825. No new matter has been added.

Attachment: Six (6) Replacement Sheets of Drawings